REMARKS

Claims 1-3, 10-14, 20, 22, 36-51 are currently pending in the application. Claims 6, 9, 18, 21, are cancelled without prejudice. Claims 1, 3, 10, 12, 14, 20, 22, 36-37, 44, 48, 50 are currently amended. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

Amendments in the Specifications

In a telephone interview of June 21, 2004, Applicants and the Examiner has agreed to incorporate the sequences for JDF-3 DNA polymerase into the present specification, although Applicants believe the specification as originally filed (i.e., without the incorporation) already satisfies the enablement requirement.

Such incorporation is supported in the specification, e.g., page 11, lines 15-16 and it is permitted under MPEP 608.01(p). Applicants respectfully request the entry of the incorporation.

Claim Rejections under 35 U.S.C. §112, First Paragraph

- ***Written Description***

Claims 6, 18 and 20 remain rejected under 35 U.S.C. §112, first paragraph. The Office Action states the claims lack written description because while applicants do not define adequately the structure to function relationship of ***any*** Pfu DNA polymerase having a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

Applicants respectfully disagree based on the same reasons as presented in our previous response filed on December 18, 2003. However, for the sole purpose of expediting the present prosecution, Applicants have cancelled claims 6 and 18 and their dependent claims 9 and 21 without prejudice. Applicants amended claims 10, 20, and 22 to depend from non-cancelled claims 1 and 12, respectively. Applicants reverse the right to prosecute the subject matters of claims 6 and 18 in a continuation application.

- ***Enablement***

Applicants wish to thank the Examiner for the withdrawal of rejections over claims 6, 18 and 20 under 35 U.S.C. §112, first paragraph, for lack of enablement.

Claims 3, 14, 44-47, and 50-51 remain rejected under 35 U.S.C. §112, first paragraph. The Office Action states that a deposit of the referred bacterial DNA polymerase is required to enable one skilled in the art to make and/or use the invention. In an interview with Applicants' representatives on June 21, 2004 (see Statement of Substance submitted herewith), Examiner Hutson clarified that the enablement rejection was issued in particular because of the recitation of the JDF-3 DNA polymerase. The Examiner stated a deposit for JDF-3, not for any other DNA polymerases recited in the claims, was required because JDF-3 DNA polymerase was not deemed readily accessible to the public.

Applicants respectfully disagree. Applicants submit that all DNA polymerases recited which can be used as the first enzyme of the present invention, including the JDF-3 DNA polymerase, are known in the art and they are readily accessible to the public. No deposit for any of the DNA polymerases is required.

First, Applicants submit that all recited DNA polymerases are well known in the art. As stated in the previous response filed December 18, 2003, the specification provides sequence accession number for each of the claimed DNA polymerase (e.g., pages 13-18). The specification further provides at least one publication reference for each of the DNA polymerases recited in the rejected claims (e.g., on page 11).

Second, Applicants submit that the recited DNA polymerases are readily accessible to the public as of the filing date of the present invention. It is routine for one skilled in the art of molecular biology to express a protein based on its known nucleotide or amino acid sequence. The instant specification specifically teaches the expression and purification of a DNA polymerase (mutant or wild-type) using a polynucleotide encoding the DNA polymerase (e.g., pages 30-31, Example 2). No undue experimentation is required for such routine expression and purification of any of the DNA polymerases recited.

In addition to the teachings of the specification, many of the DNA polymerases are commercially available and their availability is known to one skilled in the art. For example, page 11 of the present specification provides the availability of some DNA polymerases from various commercial sources. Applicants herein further provide more detailed information on the commercial availability of the DNA polymerases:

DNA polymerases	**Vendor**	**Catalog #**
Taq DNA polymerase	Stratagene, La Jolla, CA	600131, 600132, 600139
	Promega, Madison, WI	M1661, M1665, M1668, M1861, M1865
Tth DNA polymerase	Promega, Madison, WI	M2101, M2105
Tli (Vent) DNA polymerase	New England Biolabs, Beverly, MA	254S
	Promega, Madison, WI	M7101
Tgo DNA polymerase	Roche Applied Science, Indianapolis, IN	3186172
Pfu DNA polymerase	Stratagene, La Jolla, CA	600135, 600136, 600140,
	Promega, Madison, WI	M7741, M7745
KOD DNA polymerase	Novagen, San Diego, CA	71085-3
PGB-D (Deep Vent) DNA polymerase	New England Biolabs, Beverly, MA	258S, 258L

Pwo DNA polymerase	Boehringer Mannheim, Indianapolis, IN	1644947

Third, Examiner Hutson clarified during the interview that no deposit was required for any other DNA polymerases, but the claims were rejected for their recitation of "JDF-3 DNA polymerase." The Examiner felt that the JDF-3 DNA polymerase was not readily accessible to the public.

With respect to **JDF-3 DNA polymerase** particularly, Applicants submit that JDF-3 DNA polymerase was not only known in the art, but also readily accessible to the public as of the instant patent application filing date. For example, JDF-3 DNA polymerase is described on page 11 (lines 15-16) of the specification as a DNA polymerase described in patent application WO 01/32887. WO 01/32887 was published May 10, 2001 before the filing date of the instant application.

During our June 21, 2004 telephone interview, Examiner Hutson agreed with Applicants' that JDF-3 polypeptide sequence was available, but maintained that the phrase "JDF-3 DNA polymerase," as used in the claims, even in view of the teachings in the specification, did not specifically disclose the particular JDF-3 sequence which would enable the making and using of the JDF-3 DNA polymerase in the claimed invention. Although Applicants believe the above teachings satisfy the enablement requirement for JDF-3 DNA polymerase, for the sole purpose of expediting the prosecution, Applicants thereby further incorporate JDF-3 amino acid sequence (SEQ ID NO: 2 of WO 01/32887) and its corresponding DNA sequence (SEQ ID NO:1 of WO 01/32887) into the present specification. The incorporation of the sequences is permitted under MPEP 608.01(p). Examiner Hutson agreed that such incorporation would obviates the enablement rejections on claims 3, 14, 44-47 and 50-51 because of the recitation of JDF-3 DNA polymerase. During the interview of June 21, 2004, Applicants' representative, by mistake, referred to US Patent No. 5,602,011 recited on page 15 of the specification as the patent that contains the JDf-3 sequences. Applicants wish to correct the mistake and state that WO

01/32887 recited on page 11 of the present specification contains the correct JDF-3 polypeptide and nucleotide sequences.

In view of the above, Applicants submit that all DNA polymerases recited in claims 3, 14, 44-47, 50 and 51 are known in the art and are readily accessible to the public and/or they can be obtained without undue experimentation. One skilled in the art, therefore, will know how to make and use the present invention as claimed based on the teaching of the present specification. Applicants, therefore, respectfully request the 112, second paragraph on claims 3, 14, 44-47, 50 and 51 be withdrawn.

Claim Rejections under 35 U.S.C. §103(a)

Applicants wish to thank the Examiner for the removal of claims 6, 9, 18, 21, 40, 44, 49-50 from the 35 U.S.C. §103(a) rejection.

Claims 1-3, 10-14, 20, 22, 36-37, 48 and 51 remain rejected under 35 U.S.C. §103(a) as being obvious over Barns et al. (U.S. Patent No. 5,436,149) and Komori et al.

The Office Action states that, specifically, Barnes teach a formation comprising at least one thermostable DNA polymerase lacking 3'-5' exonuclease activity and at least one thermostable DNA polymerase exhibiting 3'-5' exonuclease activity, where the thermostable DNA polymerase exhibiting 3'-5' exonuclease activity is a variant of the Pfu DNA polymerase "*wherein the DNA polymerase activity of said Pfu DNA polymerase has been diminished or inactivated.*" The Office Action specifically points to claim 8 of Barnes et al. The Office Action states that "Komori et al. teach the functional interdependence of DNA polymerizing and 3'-5' exonucleolytic activities in Pfu DNA polymerase...a number of Pfu DNA polymerase mutants which affect both the DNA polymerizing and/or the 3'-5' exonucleolytic activity in varying amounts." The Office Action points out two specific Pfu mutants taught in Komori et al., i.e., D405A and D405E. The Office Action states that one skilled in the art would have been motivated to used the mutants taught in Komori et al. in the formulation disclosed in Barns et al. because Barns teach "that the ratio of the 'polymerase without 3'-exonucleaolytic activity' to the

'polymerase with 3'-exonucleolytic activity, wherein the polymerase activity is reduced or diminished' is high."

Applicants respectfully disagree and maintained that claims as previously presented are not obvious over Barnes et al. and Komori et al. However, for the sole purpose of expediting the present prosecution, Applicants have amended the claims. Applicants preserve the right of pursuing the subject matters as previously presented in claims 1-3, 10-14, 20, 22, 36-37, 48 and 51 in a subsequent continuation application.

The amended independent claims, i.e., claims 1, 12, 36, 48, as well as claims dependent from them, are drawn to an enzyme mixture comprising a first enzyme and a second enzyme, wherein said second enzyme is a **mutant Pfu DNA polymerase** comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: **Y410, T542, D543, K593, Y595, Y385, G387, and G388.**

First, neither Barnes et al. nor Komori et al. teaches or suggests the present invention as recited in claims 1-3, 10-14, 20, 22, 36-37, 48 and 51. Barnes et al. only describes a formulation with a majority DNA polymerase component lacking 3'-5' exonuclease activity (e.g., Taq DNA polymerase) and a minority DNA polymerase component exhibiting 3'-5' exonuclease activity (e.g., wild type Pfu DNA polymerase). According to Barnes et al., such formulation is useful in increasing the efficiency and accuracy of PCR amplification of long DNA targets (column 16, lines 53-45). Komori et al., the secondary reference cited, does not teach an enzyme mixture at all. Komori et al. simply teaches specific D405 Pfu mutants (i.e., D405A and D405E) that had reduced polymerase activity.

Applicants submit that the pending claims are drawn to an enzyme mixture comprising first and second enzymes. In all pending claims, the second enzyme is a mutant *Pfu* containing mutations reducing its polymerization activity. The second enzyme of the present invention as claimed, therefore, inherently exhibits 3'-5' exonuclease activity (exo^+) as known in the art and as described on page 2, lines 16-23. In some claims, e.g., claims 3, 14, 40-47, 49-50, the first enzyme is also an enzyme exhibiting 3'-5' exonuclease activity; while in other claims, e.g.,

claims 1-2, 10-13, 20, 22, 36-39, 48 and 51, the first enzyme is or may be an enzyme without 3'-5' exonuclease activity (exo$^-$).

With respect to claims drawn to an enzyme mixture containing two exo$^+$ enzymes such as claims 3, 14, 40-47, 49-50, Applicants submit that Barnes et al., the primary reference cited in the Office Action, does not teach a composition with two DNA polymerases, wherein both enzymes exhibiting 3'-5' exonuclease activities. Komori et al., the secondary reference cited, does not teach an enzyme mixture at all, therefore does not remedy the defect of Barnes et al. Barnes et al. either alone, or in combination with Komori et al., therefore, does not teach the claimed subject matter of claims 3, 14, 40-47, 49-50. During the June 21, 2004 telephone interview, Examiner Hutson agreed that the references do not make Applicants' invention obvious when the claims are drawn to an enzyme mixture containing two enzymes, both of which have 3'-5' exonuclease activity.

With respect to other claims, i.e., claims 1-2, 10-13, 20, 22, 36-39, 48 and 51, Applicants also believe that Barnes et al., either alone or in combination with Komori et al., does not make them obvious. However, for the purpose of expediting the prosecution and without acquiescing to the rejections, Applicants amended claims 1, 10, 12, 22, 36, and 48 so that they don't recite the amino acid position D405. During the telephone interview of June 21, 2004, Examiner Hutson agreed that such amendments would obviate the obviousness rejections.

Second, there is no suggestion or motivation to combine the prior art references. In order to establish a prima facie case of obviousness, there must be some reason, suggestion, or motivation from the prior art as a whole that indicates that the person of ordinary skill would have combined or modified the references. The Federal Circuit has stated:

> "[O]bviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching, suggestion or incentive supporting the combination."[1]

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[1] *In re Geiger*, 815 F.2d 686, 688, 2 U.S.P.Q.2d 1279, 1278 (Fed. Cir. 1987)

As stated above, Barnes et al. describes a formulation with a majority DNA polymerase component lacking 3'-5' exonuclease activity (e.g., Taq DNA polymerase) and a minority DNA polymerase component exhibiting 3'-5' exonuclease activity (e.g., **wild type Pfu DNA polymerase**). Barnes et al. does not teach or suggest a mutant DNA polymerase should be used as the minority DNA polymerase component in the formulation, let alone a mutant Pfu DNA polymerase comprising one or more mutations at the recited specific amino acid positions Y410, T542, D543, K593, Y595, Y385, G387, and G388.

Komori et al. studies the structure-function relationship of Pfu DNA polymerase, i.e., what mutations affect or abolish the DNA polymerase and exonuclease activities of Pfu:

> "To expand our knowledge of the structure-function relationships fo the family B DNA polymerases, and especially to understand the structural relationship between the DNA polymerizing and 3'-5' exonucleolytic activities in the polymerase protein, we prepared several mutant proteins of Pol BI from *Pfuriosus* by a unidirectional deletion strategy and site-specific mutagenesis, and analyzed their activities." (Page 41, the right column).

> "In conclusion, our mutational analysis further supports the idea that the polymerase and exonuclease domains in the family B DNA polymerases are functionally interdependent. More detailed analyses will be necessary to understand the molecular mechanism of the functional interaction between the two activities in the DNA polymerases." (Page 47, last paragraph).

Komori et al. describes two specific **D405** Pfu mutants (i.e., D405A and D405E) that have reduced polymerase activity. Komori et al. does not teach or suggests that these two mutants can be used with another DNA polymerase in the way claimed in the present invention. **In fact, Komori et al. does not teach that these two Pfu mutants can have any utilities at all.** Even without the present amendment, that is, even if the claims still recite the D405 mutation, one skilled in the art, based on the teachings of Komori et al. and absent of the teachings of Applicants' present invention, would likely avoid mutating the D405 residue of Pfu DNA polymerase to preserve its DNA polymerase and exonuclease activity.

Therefore, nothing in the two references teaches or suggests to combine or modify the references to reach to the present invention, that is, an enzyme mixture comprising a first enzyme and a second enzyme, wherein the second enzyme is a mutant Pfu DNA polymerase comprising

one or more mutations at the recited specific amino acid positions Y410, T542, D543, K593, Y595, Y385, G387, and G388. Because the prior art references fail to provide any suggestion or incentive to combine or modify the references, the Office Action fails to establish a prima facie case of obviousness.

Third, even when the prior art references are combined, they do not result in the present invention as claimed. The amended independent claims, i.e., claims 1, 12, 36, 48, as well as claims dependent from them, are drawn to an enzyme mixture comprising a first enzyme and a second enzyme, wherein said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: **Y410, T542, D543, K593, Y595, Y385, G387, and G388.** The combination of Barnes et al. and Komori et al. still does not teach or suggest an enzyme mixture comprising a mutant Pfu DNA polymerase comprising one or more mutations at the 8 specific mutations recited in the claims of the present invention.

In view of the above, Applicants submit that claims 1-3, 10-14, 20, 22, 36-37, 48 and 51 are not obvious over Barnes et al. and Komori et al. Applicants respectfully request the 103 rejections be withdrawn.

Claim Rejections under Obviousness-type Double Patenting

Claims 1-3, 6, 9-14, 18, 20-22 and 36-51 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 64-70, 75-87 of copending Application No. 10/079,241. The Office Action states that although the conflicting claims are not identical, they are not patentably distinct from each other.

While not necessarily acquiescing to the rejection, Applicants submit that they will submit a terminal disclaimer to disclaim any portion of a patent issuing from the present application which would extend beyond the term of a patent issuing from the 10/079,241 application, upon notification of allowable claims in the present application.

CONCLUSION

Applicants submit that in view of the foregoing amendments and remarks, all issues relevant to patentability raised in the Office Action have been addressed. Applicants respectfully request the withdrawal of rejections over pending claims 1-3, 10-14, 20, 22, 36-51.

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

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